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The study of biochemical parameters of Common Carp (*Cyprinus carpio* L.) juvenile in transportation using Celmanax liquid Yeast by supplementation in diet

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Abstract

The effects of Celmanax liquid commercial perbioticon biochemical parameters Common Carp (*Cyprinus carpio* L.) juvenile were studied during a long-distance transportation in plastic bags. 300 Common Carp juvenile with average weight of 30.50±1.50 g were fed by supplemented diet of Celmanax liquid commercial perbioticwith level of 1ml/kg of diet at during of 90 days and introduced to 20 liter plastic bags in stocking density of 0.1 kg/ l in 5 treatments (water temperature of 25^{oC}, 18^{oC}, 10^{oC}, water salinity of 5 and 0.5 g/l) with three replicates. In total 18 plastic bags were used in this trial. The experiment was carried out in completely random design. The effect of 12 h transport period on the biochemical profiles of common carp were investigated in experimental treatments. A quantity of 2-3 mL of blood was taken from 10 fish of plastic bag by caudal venous puncture at the beginning and the ending of the experimental trial. The aspartate aminotransferase (AST) alkaline phosphatase (ALP), alanine aminotransferase (ALT) and activities were estimated using commercial kits (Pars Azmoon, Tehran, Iran) and an Auto analyser. The level of plasma glucose of Common carp juvenile was 198.50 ng/ml in control group and was decreased to 135.00 ng/ml in treatment of A (fish were fed by Celmanax liquid commercial perbiotic supplemented diet) after 12 h transportation period at temperature of 25^{oC} , while the decrease of water temperature to 10 ^{oC} , increased this immunity factor to 203.50 ng/ml. The maximum of AST (361.50 IU/L) was obtained in treatment of C where the common carp juvenile were transported for 12 h in water of 10 ^{oC} but the minimum (129.5 IU/L) of this liver enzymes in plasma was observe in water of in treatment of A. The transportation of Carp juvenile in control group had lowest ALP (27.07 U/L). However using the Celmanax liquid commercial perbiotic in experimental treatments at transportation of Common Carp in plastic bag had variety effects on plasma biochemical parameters.

Key words: Celmanax liquid yeast, Biochemical parameters, transportation, Common Carp, cortisol

1- Introduction

In aquaculture, fish exposure to external stressors is a commonplace because of management procedures such as transporting, weighting, grading, and the increase in rearing densities. Transporting live fish is a multiphase operation that should be designed to minimize stress as well as economic costs. Water quality and fish density must be controlled. Physiological responses of fish to stressors are adaptive, resulting in mobilization of energy reserves and cardiovascular changes that enable fish to overcome disturbances [1].

Excretory products, mucus and regurgitated food also degrade the water quality. Respiration causes a decrease in the levels of dissolved oxygen and increases the levels of carbon dioxide, and excretion of nitrogenous wastes increases ammonia in the transport water [2]. Fish exposed to stressful stimuli during transport, e.g. handling, netting, loading at high densities, unloading, inadequate water exchange and poor water quality, usually suffer from a fight or flight stress response and it may cause adverse physiological reactions affecting the essential life functions [3,4]. In aquaculture, fish exposure to external stressors is a commonplace because of management procedures such as weighing, grading, transporting, and the increase in rearing densities. Physiological responses of fish to stressors are adaptive, resulting in mobilization of energy reserves and cardiovascular changes that enable fish to overcome disturbances [1].

This General Adaptation Syndrome-like stress response [5], manifests itself in a primary release of adrenalin and cortisol, followed by secondary changes in blood and tissues, such as hyperglycaemia, hyperlactaemia, hypercholesterolaemia, changes in blood plasma enzyme activities and ion concentrations, reduced glycogen content of muscle and liver, increased metabolic rate, and shifts in hematological profiles and immunological capacity [6,7]. The acute primary physiological response of fish to netting, handling and transport returns to normal levels within 6-24 h. However, physiological recovery may take 10–14 days if the stressors persist and are not lethal [8]. Stress and physical activity during transport usually lead to the loss of product quality, such as reducing fish freshness, softening muscle texture, and lowering filet yield. Exposure to stress can have an impact on the economics of fish aquaculture [9].

Yeast have been identified as part of the normal microbiota of both wild and farmed fish, and their role in fish health and nutrition has been addressed in the literature, as yeast have been used either alive to feed live food organisms or after processing as a feed ingredient after demonstrating an artificial colonization of the intestinal host. Yeasts are widely distributed in several natural environments such as soil, freshwater, and seawater. Their numbers and species distributions are dependent on the concentrations and types of available organic materials.

The improvement of the immune response is one of the most encountered side effects in the host because immune system stimulation or immunomodulation are considered important mechanisms supporting probiosis. Yeast have immunostimulatory properties because they possess components such as β-glucan, mannoproteins, chitin (as a minor component) and nucleic acids [10].

Recent studies have shown the beneficial effect of dietary administered Saccharomyces cerevisiae in fish. Yeast supplemented diets stimulate growth, feed efficiency, blood biochemistry, survival rate, and non- specific immune responses in Uronema marinum-infected olive flounder (Paralichthys olivaceus) [10]. A diet supplemented with S. cerevisiae treated with beta-mercaptoethanol was better than whole cell yeast and n-3 highly unsaturated fatty acids (HUFA)- enriched yeast as an immune system and growth stimulator in juvenile rainbow trout challenged with Yersinia ruckeri [11]. Similarly, the dietary administration of the probiotic S. cerevisiae P13 at a minimum level of 105 CFU/kg enhanced the growth, innate immune responses and disease resistance of grouper (Epinephelus coioides)[12].

The aim of this study was to evaluation of yeast of Celmanax liquid as a commercial product in transport stress response, i.e. the effect of manipulation of physical condition of water in plastic bags by changes of temperature and addition of salt (NaCL) for transportation to some biochemical factors in blood serum of Common Carp (*Cyprinus carpio* L.) juvenile.

2-Material and methods

Fish biomass used in this study was represented by Common Carp juvenile (*Cyprinus carpio* L.) with an average weight (±SD) of 30.50±1.50 g, raised into a race way system of the pilot aquaculture station (fiberglass tank with total volume of 3 m^3 and continuous aeration).

The Common Carp juvenile were purchased form fish commercial warm water farm of Chamran, (Golestan province –Iran) and acrid to aquaculture laboratory of Gonbade Kavos university.

Fish were acclimatized in laboratory condition for 10 days. For this experiment, the feeding intensity was of 5% per body weight per day (for the first ten days), in two meals per day. The food used was extruded pellets from Faradaneh Co. (Iran) with the diameter of 1 mm and with 50% protein content.

The yeastof)*Saccharomyces cerevisiae)* under the commercial title of Celmanaxliquid was preparedfrom Arm & Hammer Animal Nutrition Co. (USA).

The Celmanax liquid commercial perbioticsuspended at level of 1 ml per 1000 ml of water. This suspension was added to trout fed if FFT1 and mixed with it. This experimental diet was dried in 40 ^{oC} at 4 hours. The Common Carp juvenile were fed by supplemented diet with concentration of 1 g/kg of diet in three times a day at 5-7 percentage of body mass. In control group the Common Carp juvenile were fed by non supplemented diet with Celmanax liquid yeast.

Briefly, Common Carp juvenile were placed in double layered 40 L plastic bags filled with fresh water (volume 20 liter) and 20 fish (weight of average weight of 100.20±0.22 g or 2 kg/ 20 L, inflated with oxygen (capacity of 20 liters), and tied with rubber-bands. The bags were then placed in Styrofoam boxes. In treatment of A, B and C the plastic bags were gently filled by water with temperature of 25 ^{oC}, 18^{oC} and 10 ^{oC} respectively and kept in these temperature for 12 h. Also in experimental treatment of D and E in water of plastic bags were added the NaCl in levels of 5 and 0.5 g per liter respectively. The fish of control group were transported in plastic bags were gently filled by water with temperature of 25 ^{oC}.

After 12 h, in final of experiment, a quantity of 2-3 mL of blood was taken from 10 fish of plastic bag by caudal venous puncture using heparin as anticoagulant at the beginning and the ending of the experimental trial.

The serum of blood samples were aliquoted into non-heparinized tubes and left to clot for 12 h (at 4^{oC}), prior to centrifugation at 5,000g for 5 min at 4^{oC} in a clinical centrifuge (Hettich-D7200, Tuttlingen, Germany). Isolated serum was stored at -20^{oC}. Glucose levels were determined by the glucose oxidase methods using enzymatic-colorimetric methods by means of commercial kits[13]. Cortisol serum concentration was measured by ELISA using anti-cortisol-3-CMO-BSA (FKA-404, Cosmo Bio, Tokyo, Japan) and labeled-steroid cortisol-3-CMO-HRP (FKA-403) according to the method of Asahina et al. (1995) [14]. Plasma chloride and calcium were measured using a colorimetric method using Technicon autoanalyser (Technicon Instruments Corporation, New York, USA).

Serum, aspartate aminotransferase (AST) alkaline phosphatase (ALP), alanine aminotransferase (ALT) and activities were estimated using commercial kits (Pars Azmoon, Tehran, Iran) and an Auto analyser (Eppendorf, EPOS, Germany) after Shahsavani et al. (2010) [15].

Data are expressed as mean values ± SD. All data were subjected to one-way analyses of variance (ANOVA). If significant differences were indicated at the 0.05 level, then a multiplecomparisons (Duncan's multiple) test was used to examine significant differences among treatments using SPSS computer software. Statistical significance required that $p<0.05$ [16].

3- Results

Results of plasma biochemical profile of experimental groups of common carp exposed to different condition after 12 h transportation period (table 1) showed a significant difference in plasma glucose ($p < 0.05$), and a cortisol levels.

The highest level of plasma cortisol (66.30 ng/dl) in common carp juvenile were obtained in control group and in another experiment treatment this immunity factor was significantly decreased ($p > 0.05$). Also the lowest of this plasma parameter (11.60 ng/dl) was showed in experimental treatment of B, where the common carp juvenile fed with supplemented diet by Celmanax liquid and were transported in plastic bag with water temperature of 18^{oC}.

In control group the level of plasma glucose of Common carp juvenile after 12 h period of transportation in plastic bags with temperature of 25^{oC} was 198.50 ng/ml but in treatment of A (fish were fed by Celmanax liquidsupplemented diet) was decreased to 135.00 mg/ml. The highest level of plasma glucose (203.50 mg/ml) in common carp were observed in treatment of C. In this treatment the common carp juvenile were fed by supplemented diet with Celmanax liquid and were transported for 12 h in water of 10^{oC} in plastic bags.

The maximum of AST (361.50 IU/L) was obtained in treatment of C where the common carp juvenile were transported for 12 h in water of 10^{oC} but the minimum (129.5 IU/L) of this liver enzymes in plasma was observe in water of in treatment of A. The transportation of Carp juvenile in control group had lowest ALP (27.07 U/L). The treatment of A had significant difference with control group and another experimental treatment ($p > 0.05$).

Table1. The immunity factors and enzymes of Common Carp (*Cprinus carpio*) juvenile fed with supplemented diet with Celmanax liquid in different treatments and control

Treatment / parameter	Control25 ^{oC}	A25 ^{oC}	B18 ^{oC}	C10 ^{oC}	D5g/l	E0.5g/l
Cortisol (ng/dl)	66.30±3.15 ^{a}	21.05±3.55 ^{b}	11.60±2.80 ^{b}	25.95±1.45 ^{b}	28.35±0.15 ^{b}	27.70±5.70 ^{b}
Glucose (mg/dl)	198.50± 29.50 ^{a}	135.00± 8.00 ^{a}	189.20±25.50 ^{a}	203.50±53.50 ^{a}	163.00±47.00 ^{a}	196.00±37.00 ^{a}
Calcium (mg/dl)	10.60± 0.40 ^{ab}	10.50± 0.20 ^{b}	10.40±0.60 ^{b}	11.45±0.75 ^{a}	10.60±0.04 ^{ab}	10.60±0.50 ^{ab}
A.S.T.(SGOT) (IU/l)	150.00±23.00 ^{b}	129.50±9.50 ^{b}	166.50±58.5 ^{b}	361.50±80.85 ^{a}	190.00±56.85 ^{b}	139.50±1.50 ^{b}
A.L.T.(SGPT) (IU/l)	50.00±5.00 ^{a}	8.00±2.01 ^{c}	11.00±4.00 ^{bc}	23.50±4.51 ^{b}	14.03±2.05 ^{bc}	22.06±4.00 ^{b}
ALP (IU/l)	27.07±1.06 ^{b}	145.50±31.50 ^{a}	157.50±29.50 ^{a}	133.50±30.50 ^{a}	147.08±10.01 ^{a}	137.50±2.50 ^{a}

The concentration of Calcium in plasma didn’t differ statistically among the experimental treatment tested ($p > 0.05$).

Table1. The immunity factors and enzymes of Common Carp (*Cprinus carpio*) juvenile fed with supplemented diet with Celmanax liquid in different treatments and control

The values of plasma alanine aminotransferase (ALT) were significantly influenced by the Celmanax liquidin processing of transport of Common carp juvenile in plastic bags. All of experimental treatments had the lower levels of plasma ALT in comparison with the plasma ALT of Common carp juveniles at the control group. The highest of this liver enzyme was observed in control group (50.00 IU/L) where the Carp juveniles were transported in plastic bags with water salinity of 25 ^{oC} and fed by non supplemented diet with Celmanax liquid. The experimental treatments of C and E had significantly difference with control and treatment of A ($p < 0.05$).

Regarding the biochemical profiles (table1) the Serum, alkaline phosphatase (ALP) of Common carp juvenile in plastic bags significantly changed, but independently using Celmanax liquidand water condition in transportation period. This plasma factor was increased in treatment of A (145.50 U/L), B (157.50 U/L), C (133.50 U/L), D (147.08 U/L) and E (137.50 IU/L) was significantly decreased in experimental treatments in comparison with the plasma ALP of Common carp juveniles at the control group ($p < 0.05$).

4- Discussion

The transporting process is a stress factor for cultivable fishes. Commonly a long transportation period is stressful events for fishes and increases the biochemical factor of stress in blood Plasma.

Bakers’ yeast, *Saccharomyces cerevisiae*, is used for the bakers’ industry that contains various immunostimulating compounds such as β-glucans, nucleic acids as well as mannan oligosaccharides, and it has the capability to enhance immune responses [17] of various fish species. However, the administration of yeast has been recognized to have important effects on immunostimulant functions[18].

Yeast β-glucans have been applied in aquaculture to modulate the innate immune system of fish to improve their survival until adaptive immune responses are sufficiently developed to mount effective responses against pathogens.

There aren't any reports for using yeast extract for decreasing stress in transportation of common carp in plastic bags. In generally adding of salt and decline of temperature using for decreasing of stress in fish during transportation. Addition of salt into the water of plastic bags in transporting period decreased the stress of Common Carp (*Cprinus carpio*) juveniles. But in the present study, the supplementation of commercial *S. cerevisiae* of Celmanax liquid, improved the biochemical parameters of Common Carp (*Cyprinus carpio* L.) juvenile during a long-distance transportation in plastic bags.

The use of optimum level of salt during transportation helps fish to maintain homeostasis by reducing the osmotic gradient between plasma and external environment and by increasing mucus production [19]. which has been demonstrated to reduce stress responses for some cultivable fish species. Similar results were obtained by Barton and Peter (1982) for rainbow trout (*Oncorhynchus mykiss*) [20] and matrinxã (*Brycon cephalus*) [21], but in accordance with their findings has not been tested for pirarucu transportation. Therefore this study analyzed several parameters (blood glucose and cortisol) in an attempt to evaluate the effect of salt on physiological homeostasis of Common Carp (*Cprinus carpio*) juveniles during transportation of juveniles in plastic bags.

Decreasing of plasma cortisol and glucose in juvenile carp during the transportation of Common Carp in plastic bags. In agreement with our results, He et al. (2008) observed using dietary *Saccharomyces cerevisiae* fermentation product (DVAQUA) had a best effects on non-specific immunity of hybrid tilapia (*Oreochromis niloticus* ♀×*O. aureus* ♂) cultured in cages[22].

When fish are stressed, their adrenergic response releases adrenaline and noradrenaline into the

blood stream and a hypothalamo-pituitary-interrenal response ultimately leads to an increase in the plasma cortisol level [23]. In all vertebrates, including fish, cortisol plays a key rolein the restoration of homeostasis during and/or after stress [24]. However, quantifying stressful conditions proved difficult with many authors reporting a transient increase in plasma cortisol levels during stress, e.g. during crowding [25,1], while others reported no effect (Procarione et al., 1999) or reduced cortisol levels [26].

The plasma calcium level was increased in transportation period at temperature of 10^{oC}. But didn't significant difference with plasma calcium in another experimental treatments. Mazeaud et al. (1997) and Schreck (1990) reported that the stress leads to a hydromineral imbalance in plasma and rectification of the stress- related osmotic dysfunction places an energetic load on the fish [27,28]. In addition, electrolytes serve as a general measure of osmoregulatory dysfunction [29].

Hematological profiles have often been used as stress indicators. Major shifts in the hemogram are found in fish exposed to acute or chronic stress. In the present study, serum AST, ALT and ALP levels were affected by of transportation in long time (12 h.). The assessment of multiple enzymes is considered a valuable tool for indications of stress, tissue damage, which can complement microbiological, histopathological techniques [30]. To our knowledge, very little information is available regarding serum enzymes in fish transprtation; however, Dobsikova te al., (2006) reported long distance transportation had significant effects on these enzymes in Common carp [31].

The feeding of common carp juvenile by supplemented diet with Celmanax liquid concentrate had best results in decreasing the liver enzymes of AST and ALT in plasma of juvenile carp during a long-distance transportation in plastic bags.

On the other hand, these researchers were showed the transportation of fish is a stressful factor to increasing of these liver enzymes in fishes. This confirmed the results obtained in the our studies but the supplementation of Celmanax liquidin diet of common carpdecreased this liver enzymes levels in plasma of Common carp juvenile and increasing the health rate and welfare of this fish.

Plasma glucose is elevated in stressed fish as a consequence of increased blood catecholamine [32]. The elevation of plasma lactate follows respiration under anaerobic conditions and extreme exercise [33]. In our study, glucose was high in fish in water with temperature of 10^{oC}. This may be attributed to cold stress in conditions of transportation period. The glucose level in control group of carp was in high level, 198.50 mg/l; similar results were reported by Hertz et al. (1989) and Pottinger (1998). They reported at the end of the transport, a significant decrease in ALT ($p < 0.05$), ALP ($p < 0.01$) and chloride ($p < 0.05$) levels, and a significant increase in ammonia ($p < 0.01$) concentration were found. Initial high ALT, ALP and chloride levels may be ascribed to the enhancement of fish basal metabolism during stressful reloading. Subsequent modulation of fish during transport leads to a decrease in their levels[33,34]. Davison et al. (1994) reported that hypoxic conditions caused an increase in chloride levels in fish [35].

In conclusion this study showed that the different condition of water in plastic bag at transportation of Common Carp juvenile had variety effects on plasma biochemical parameters and supplementation of Celmanax liquidyeast of *Saccharomyces cerevisiae* in diet of common carp, had the better results on decreasing of liver enzyme, cortisol and increasing immunity of Common Carp juveniles during transportation.

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